

CLAIMS:

1. A novel molecule useful for anthrax toxin inhibition in vivo comprising a recombinant protein designated as PA-I, which is a dominant negative inhibitor of PA, wherein the 2 β 2-2 β 3 loop comprises amino acid residues of the amphipathic loop of the homologous toxin iota-comprising:

'iota b toxin' sequence inserted at 2 β 2-2 β 3 loop in the recombinant PA-I:

³⁰² V G V S I S A G Y Q N G F T G N I T T S A G F³²⁴ SEQ 2

2. The mutated gene encoding the recombinant protein with sequence of base pairs at 2 β 2-2 β 3 loop comprising:

GTA GGA GTT TCA ATT TCA GCA GGG TAT CAG AAC GGC TTT ACT GGT
AAT ATC ACT ACA TCT GCA GGA TTT

3. The oligonucleotide primers encompassing the mutation:

Primer 1:

5'< ATT ACT AAA TCC TGC AGA TGT AGT GAT ATT ACC AGT AAA GCC GTT
CTG ATA CCC TGC TGA AAT TGA AAC TCC TAC AGT ATT AGC ATC CCT
ACT TGT AGA AGT ATT TTT AC> 3'

Primer 2:

5'< GT GAT TAA TAA AGC TTC TAA TTC > 3'

4. A method for developing the novel recombinant anthrax toxin inhibitor protein as claimed in claim 1, said method comprising steps of:

- a. designing the oligonucleotide primers encompassing the mutation site comprising:

Primer 1:

5'< ATT ACT AAA TCC TGC AGA TGT AGT GAT ATT ACC AGT AAA GCC GTT
CTG ATA CCC TGC TGA AAT TGA AAC TCC TAC AGT ATT AGC ATC CCT
ACT TGT AGA AGT ATT TTT AC> 3'

Primer 2:

5'< GT GAT TAA TAA AGC TTC TAA TTC > 3'

- b. amplifying a portion of the PA gene encoding the mutant region of the 2 β 2-2 β 3 loop,

- c. cloning the amplified fragment back into the plasmid and inserting the plasmid into *Bacillus anthracis* for expression of the mutated gene,
 - d. purifying the mutant protein from the culture supernatant of *B. anthracis* followed by characterization of the expressed mutant protein,
 - e. checking the cytotoxicity of the expressed mutant on mammalian cells in vitro.
 - f. testing the inhibiting ability of the mutant protein for inhibiting the toxic activity of native PA when present at equimolar or lower concentrations,
 - g. assaying for the ability of the mutant protein to inhibit pore-forming ability of native PA in vitro, and
 - h. testing the ability of the mutant protein to inhibit anthrax toxin activity in vivo on administration to in-vivo systems in equimolar ratio with wild-type PA plus LF.
5. A method as claimed in claim 4 wherein the strain used is selected from *E. coli* or *Bacillus anthracis*.
 6. A method as claimed in claim 4 wherein the vector for cloning the mutant gene is selected from any expression vector such as plasmid pYS5, pYS6 and pMS1.
 7. A method as claimed in claim 4 wherein the host for expressing the mutant gene is selected from *E. coli*, *Bacillus* sp. and the like or a yeast.
 8. A method as claimed in claim 4 wherein the mammalian cell lines used are selected from CHO-K1, J774A.1 and RAW 264.7.
 9. A method as claimed in claim 4 wherein the concentration of PA –I used for testing anthrax toxin inhibition is in the range of 0.01 µg/ml to 0.1 µg/ml.
 10. A method as claimed in claim 4 wherein in vivo system is used to test the in vivo anthrax toxin inhibitory effect can be Fischer 344 rats, guinea pigs, mice and the like.
 11. A method for developing the novel recombinant anthrax toxin inhibitor protein as claimed in claim 1, said method comprising steps of:
 - i) mutagenesis using the PCR primers of claim 3,
 - ii) cloning the amplified mutated fragment into the appropriate vector and inserting it into an appropriate host for expression of the mutated gene,
 - iii) purifying the mutant protein followed by characterization of the expressed mutant protein,

- [illegible]